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Synthesis and Evaluation of Indatraline-Based Inhibitors for Trypanothione Reductase

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The search for novel compounds of relevance to the treatment of diseases caused by trypanosomatid protozoan parasites continues. Screening of a large library of known bioactive compounds has led to several drug-like starting points for further optimisation. In this study, novel analogues of the monoamine uptake inhibitor indatraline were prepared and assessed both as inhibitors of trypanothione reductase (TryR) and against the

parasite *Trypanosoma brucei*. Although it proved difficult to significantly increase the potency of the original compound as an inhibitor of TryR, some insight into the preferred substituent on the amine group and in the two aromatic rings of the parent indatraline was deduced. In addition, detailed mode of action studies indicated that two of the inhibitors exhibit a mixed mode of inhibition.

Introduction

Trypanosomatid protozoan parasites cause a variety of important diseases, including human African sleeping sickness, Chagas disease, and leishmaniasis. Sleeping sickness is caused by *Trypanosoma brucei* ssp., and is endemic in certain regions of sub-Saharan Africa, covering about 20 countries with an estimated 70–80 000 people infected.^[1] Chagas disease is present in 19 countries on the American continent and is responsible for disease in around 8–11 million people.^[1] The causative agent of this disease is the parasite *Trypanosoma cruzi*. Leishmaniasis is caused by members of the genus *Leishmania* and is found in 88 countries, threatening 350 million people.^[1] In combination, these diseases contribute to approximately 95 000 deaths annually, but considering their socioeconomic importance, the current scale of new drug discovery and vaccine development efforts is inadequate. In addition, the available therapies are often toxic, marginally effective, administered by injection, and expensive. In particular, there is an urgent need for new CNS-active drugs to treat late-stage sleeping sickness to replace the current therapies that are losing efficacy due to parasite resistance.^[1]

The trypanosomatids use a polyamine–glutathione adduct, trypanothione (**1**, Figure 1), as a key component of their defence system. Compound **1** is prepared through a unique biosynthetic pathway in which glutathione (**2**) is conjugated to spermidine.^[2] In humans, glutathione and glutathione reductase (GR) are used to maintain the intracellular redox balance, whereas the analogous chemistry in the parasite is carried out by trypanothione reductase (TryR), which reduces trypanothione disulfide (T[S]₂) to **1**. Previous genetic knockout studies have illustrated the essential role of TryR in parasite viability,^[3] validating it as a target for drug development in all three diseases. Importantly, comparison of TryR and human GR crystal structures reveal significant differences between their active sites,^[4] suggesting that these differences may be exploited to gain selectivity for TryR over GR.

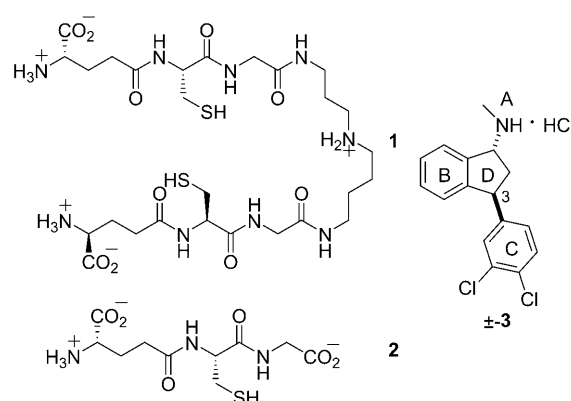


Figure 1. Structures of trypanothione (**1**), glutathione (**2**), and indatraline (**3**).

As part of a concerted campaign to discover new treatments for trypanosomatid-based diseases, we undertook a high-throughput screen for inhibitors of TryR. The Sigma-LOPAC¹²⁸⁰

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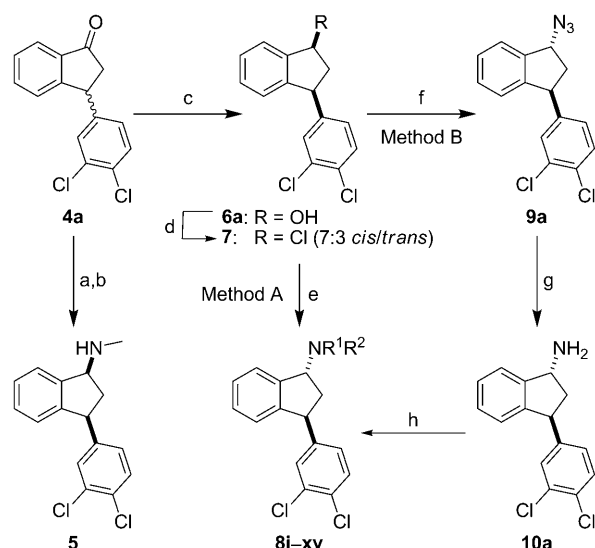
collection, a library of compounds with known pharmacological activity, was screened against TryR.^[5] The thinking behind screening a library of known drugs is encapsulated in Sir James Black's famous quote: "The most fruitful basis for the discovery of a new drug is to start with an old drug".^[6] It was planned that hits derived from small molecules that already have desirable drug-like properties could be modified to tune their selectivity away from their original protein targets and towards TryR without too much disruption of the desirable drug-like properties.

As reported previously,^[5] assessment of initial screening hits against human GR and *T. brucei* cells together with in silico analysis of chemical properties revealed three new classes of TryR inhibitors that merited further development. Investigation of one of these classes, based on 1-[1-(2-benzo[*b*]thienyl)cyclohexyl]piperidine (BTCP), was reported previously.^[7] Herein we describe a related investigation of a further class of compounds based on indatraline (**3**), a nonselective monoamine reuptake inhibitor.^[8] The molecule contains an indanamine core structure with a second aromatic unit at the 3-position (Figure 1). This CNS-active molecule was considered suitable for extensive modification and was of sufficiently low molecular weight to allow adherence to Lipinski's rules, even following considerable alteration.^[9] We identified four possible sites (A–D, Figure 1) for modification of the core structure. Details of the parallel synthetic routes that were developed along with careful analysis of the activity of the analogues generated against TryR in vitro and *T. brucei* in culture are reported. Whilst it proved difficult in this chemical series to improve potency against the desired target, a new important insight into the mode of inhibition of TryR by these analogues was discovered, progressing our thinking on how to inhibit effectively this important enzyme.

Results and Discussion

Synthesis of indatraline analogues

Initial studies focused on the amino substituent in **3** (site A, Figure 1) starting from the common intermediate 3-phenylindanone (**4a**, Scheme 1). Compound **4a** was prepared according to published methods.^[10] Treatment of **4a** with methylamine in the presence of titanium tetrachloride followed by reduction of the resulting imine with sodium borohydride afforded indanamine **5** as the *cis* isomer, as reported by Bøgesø et al.^[8a] Access to the *trans*-indanamines was achieved as follows.^[8a] Reduction of indanone **4a** with sodium borohydride gave 3-phenylindan-1-ol (**6a**) in high yield and high *cis* selectivity (97:3). A single recrystallisation was required to afford the pure *cis* isomer. Reaction of **6a** with thionyl chloride resulted in an isomeric mixture of *cis*- and *trans*-1-chloro-3-phenylindanes (**7**), with a *cis/trans* ratio of 7:3. Crude **7** was then reacted with a series of primary and secondary alkylamines to produce the corresponding 3-phenylindan-1-amines with, as expected, a reversal of the *cis/trans* ratio (3:7). The pure *trans* isomers **8i–vi** were isolated following purification by semi-prepa-

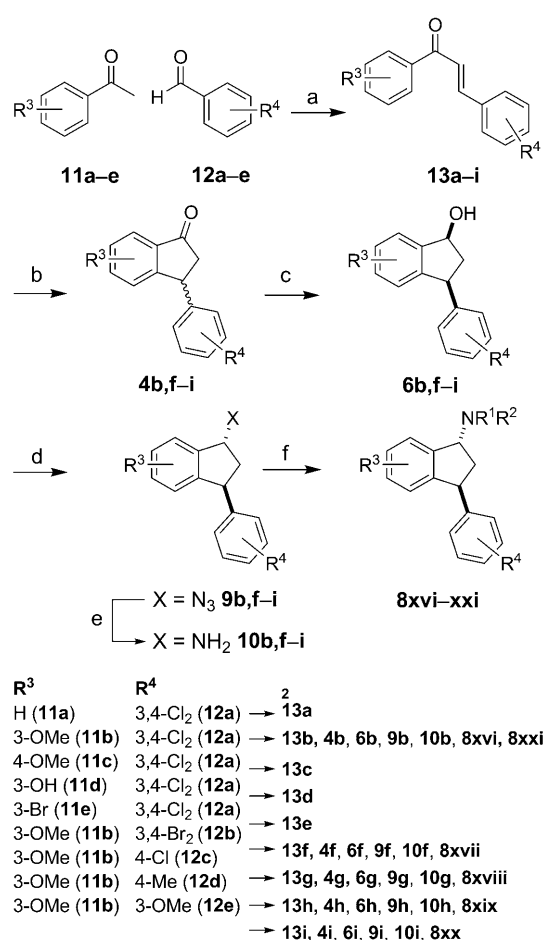


Scheme 1. Reagents and conditions: a) MeNH₂, TiCl₄, PhMe, –10 °C, 1 h; b) NaBH₄, MeOH, RT, 3 h (62%); c) NaBH₄, MeOH, RT, 2 h (77%); d) SOCl₂, Tol., RT, 3 h; e) NHR¹R², THF, 90 °C, 4 h; f) (PhO)₂P(O)N₃, DBU, THF, RT, o/n (93%); g) PS–PPh₃, H₂O, THF, RT, 16 h (quant); h) R¹CHO, NaBH(OAc)₃ or CH₃COCl or TsCl, THF, RT, o/n. R¹ and R² are defined in Table 1 and Supporting Information.^[11]

rative HPLC, and the stereochemistry was assigned by comparison with published work.^[8a]

Having prepared analogues **5** and **8i–vi**, we decided to evaluate the routes used for conversion into a parallel synthesis protocol. This was viewed as challenging due to the required separation of the isomeric mixtures of **8** on a small scale. We therefore decided to adapt the original route by incorporating a modified Mitsunobu protocol to convert indanol **6a** to the azide **9a** (Scheme 1).^[12] This reaction occurred with complete inversion of the C1 stereochemistry in **6a** and generated exclusively the *trans* isomer of **9a**, removing the requirement to separate isomeric mixtures later in the route. A Staudinger reduction of azide **9a** using polymer-supported triphenylphosphine generated the indanamine **10a** in high yield and purity following filtration to remove the reagent. Reductive amination of **10a** with a range of aldehydes in the presence of sodium triacetoxyborohydride afforded the required *trans*-indanamines **8vii–xiii**. Initially, purification of these compounds was necessary, as over-alkylation of **10a** was observed. However when an excess of **10a** (1.2 equivalents) was used in this reaction, none of the dialkylated product was formed, and remaining **10a** was removed using a polymer-supported benzaldehyde scavenger resin. With this protocol it was possible to prepare pure *trans*-indanamines **8vii–xiii** in moderate to high yields. Indanamine **10a** was also treated with acetyl and tosyl chloride to afford **8xiv** and **8xv**, respectively. The stereochemistry of **8** and **9** prepared by this route was assigned based on literature precedent from Rice and co-workers.^[12]

Having developed a robust route to amino-substituted analogues, we decided to investigate modification of the two aromatic rings present in **3**. Rice and colleagues reported a protocol to access the indanone core via an aldol condensation of 3-methoxyacetophenone (**11b**, Scheme 2) with 3,4-dichloro-



Scheme 2. Reagents and conditions: a) R⁴CHO, KOH, EtOH, 0 °C, 2 h; b) TFA, 120 °C, microwave, 10 min; c) NaBH₄, MeOH, RT, 2 h; d) (PhO)₂P(O)N₃, DBU, THF, RT, o/n; e) PS-PPh₃, H₂O, THF, RT, 16 h; f) R¹CHO, NaBH(OAc)₃, THF, RT, o/n. R¹–R⁴ are defined in Table 1 and Supporting Information.^[11]

benzaldehyde (**12a**) to generate chalcone (**13b**).^[12,13] In their hands, a subsequent Nazarov cyclisation in the presence of trifluoroacetic acid afforded 6-methoxyindanone (**4b**) in high yield. This route was particularly appealing, as it allowed rapid access to the indanone core in two steps. Furthermore, the product from the aldol reaction crystallised out of the reaction solution and required no further purification. These factors made this approach potentially adaptable to a high-throughput synthesis format. To investigate this further, a series of chalcones (compounds **13a–e**) were prepared from the aldol reaction between acetophenones **11a–e** and 3,4-dichlorobenzaldehyde (**12a**). The chalcones **13a–e** were then submitted to cyclisation reactions using trifluoroacetic acid. The Nazarov reaction only occurred when a 3-methoxy substituent was present in the chalcone (for substrate **13b**). In this case, it was possible to decrease the reaction time considerably by carrying out the reactions in a microwave reactor as opposed to conventional heating (10 min versus 4 h, respectively). Having determined the requirements for a successful Nazarov reaction, a collection of indanones (**4b,f–i**) was prepared by using 3-methoxyacetophenone (**11b**) and a variety of benzaldehydes

12b–e. The electronic nature of the substituent on the benzaldehydes did not influence the outcome of the Nazarov cyclisation, and all the chalcones afforded the desired indanones **4f–i**. These were then converted into the indanamines **8xvi–xxi** via method B (Scheme 1).

Biological analysis against TryR, GR, and *T. brucei*

The analogues prepared according to the methods described above were tested against *T. cruzi* TryR and in an assay for *T. brucei* cell proliferation, as described below in the Experimental Section.

The biological data for a selected group of these compounds are summarised in Table 1. The *cis* isomer **5** (Table 1, Entry 1) was less active against TryR than indatraline **3**, Entry 2). This resulted in the synthetic efforts being focused on the generation of *trans* analogues. As the size and lipophilicity of the amino substituent was increased (Entries 1 and 3–6), there appeared to be a relatively flat SAR (see also analogues **S8xxii–S8xxvii** in the Supporting Information^[11]). This suggests that this region of the molecule contributes little to the biological activity and is probably incorporated in a large open region of the active site. The slightly lower activity of the *N*-tBu analogue **8iii** may, however, indicate some steric constraint close to the nitrogen atom. In addition, the piperidine (**8v**, Entry 7) and morpholine (**8vi**, Entry 8) analogues were prepared. Whilst the piperidine analogue **8v** retained activity, the insertion of an additional oxygen atom, in the case of the morpholine analogue **8vi**, caused a significant decrease in activity (see also analogue **S8xxx** in the Supporting Information^[11]). By replacing the methyl substituent of indatraline with a benzyl group (**8vii**, Entry 9), the activity was retained. This provided the opportunity to synthesise a Topliss series of analogues.^[14] Therefore the 4-chloro (**8viii**, Entry 10), 3,4-dichloro (**8ix**, Entry 11), 4-methyl (**8x**, Entry 12), and 4-methoxy (**8xi**, Entry 13) analogues were prepared. The incorporation of electron-donating substituents led to the highest activity, whereas activity diminished when electron-withdrawing groups were introduced. This result led to the preparation of the remaining two compounds in the electron-rich Topliss series: 4-dimethylamino (**8xii**, Entry 14) and 4-amino (**8xiii**, Entry 15). These results further highlight the benefits associated with the inclusion of an electron-donating substituent, although no further improvement in activity was observed relative to the 4-methoxy analogue **8xi**. For data on additional substituted benzyl analogues, see table S1, analogues **S8xxxi–S8xxxv** (Supporting Information). The acetyl (**8xiv**, Entry 16) and tosyl (**8xv**, Entry 17) analogues were inactive against TryR (see also analogue **S8xxxvi** in the Supporting Information^[11]).

We decided to use the isoamyl group (see **8ii**, Entry 4) as the substituent for site A, whilst an exploration of the B and C rings was carried out. The inclusion of a 6-methoxy substituent in the B ring was investigated, as this was a synthetic requirement for the Nazarov reaction. Analogue **8xvi** (Entry 18) was prepared as a direct comparison with **8ii** (Entry 4). The increased activity of **8xvi** relative to **8ii** demonstrated that the presence of an electron-donating substituent in the B ring is

Table 1. Biological data for selected indatraline analogues.

Entry	Compound	R ¹	R ²	R ³	R ⁴	TryR IC ₅₀ [μM] ^[a]	<i>T. brucei</i> EC ₅₀ [μM]
	Pentamidine ^[b]						0.0037 ± 0.0001
1	3	Me	H	H	3,4-Cl ₂	8.84 ± 0.24	1.06 ± 0.05
2	5 ^[c]	Me	H	H	3,4-Cl ₂	13.5 ± 0.7	1.50 ± 0.23
3	8 i ^[b]	Et	H	H	3,4-Cl ₂	7.19 ± 0.40	2.12 ± 0.06
4	8 ii	Isoamyl	H	H	3,4-Cl ₂	4.05 ± 0.39	1.08 ± 0.07
5	8 iii ^[b]	tBu	H	H	3,4-Cl ₂	15.3 ± 1.4	0.48 ± 0.04
6	8 iv	Octyl	H	H	3,4-Cl ₂	7.93 ± 0.78	0.66 ± 0.04
7	8 v ^[b]	–CH ₂ (CH ₂) ₃ CH ₂ –		H	3,4-Cl ₂	5.47 ± 0.32	1.31 ± 0.11
8	8 vi	–(CH ₂) ₂ O(CH ₂) ₂ –		H	3,4-Cl ₂	173 ± 56	6.62 ± 0.77
9	8 vii ^[b]	Benzyl	H	H	3,4-Cl ₂	8.04 ± 0.91	3.53 ± 0.35
10	8 viii	4-Cl-Bn	H	H	3,4-Cl ₂	17.6 ± 3.3	3.22 ± 0.26
11	8 ix	3,4-Cl ₂ -Bn	H	H	3,4-Cl ₂	45.9 ± 4.2	8.87 ± 0.60
12	8 x	4-Me-Bn	H	H	3,4-Cl ₂	13.0 ± 1.6	1.29 ± 0.04
13	8 xi	4-OMe-Bn	H	H	3,4-Cl ₂	4.14 ± 0.41	2.36 ± 0.22
14	8 xii	4-Me ₂ N-Bn	H	H	3,4-Cl ₂	7.38 ± 1.21	1.98 ± 0.23
15	8 xiii	4-NH ₂ -Bn	H	H	3,4-Cl ₂	4.94 ± 0.43	2.68 ± 1.04
16	8 xiv	Acetyl	H	H	3,4-Cl ₂	> 200	3.64 ± 0.28
17	8 xv	Tosyl	H	H	3,4-Cl ₂	> 200	4.33 ± 0.33
18	8 xvi	Isoamyl	H	6-OMe	3,4-Cl ₂	2.23 ± 0.66	1.26 ± 0.13
19	8 xvii	Isoamyl	H	6-OMe	3,4-Br ₂	3.15 ± 0.23	2.47 ± 0.25
20	8 xviii	Isoamyl	H	6-OMe	4-Cl	15.8 ± 1.6	2.51 ± 0.33
21	8 xix	Isoamyl	H	6-OMe	4-Me	32.9 ± 4.3	1.49 ± 0.08
22	8 xx	Isoamyl	H	6-OMe	3-OMe	125 ± 17	6.99 ± 1.55
23	8 xxi	Benzyl	H	6-OMe	3,4-Cl ₂	3.07 ± 0.45	2.30 ± 0.22

[a] Inhibition of trypanothione reductase (TryR). [b] Pentamidine was included as a reference drug in biological testing.^[16a] [c] See Ref. [11].

favourable. Therefore a collection of analogues was synthesised containing the 6-methoxy substituent but with variations in substituents in the C ring. The 3,4-dibromo (**8 xvii**, Entry 19), 4-chloro (**8 xviii**, Entry 20), 4-methyl (**8 xix**, Entry 21), and 3-methoxy (**8 xx**, entry 22) substituted analogues were prepared and tested. A clear preference for electron-withdrawing substituents in the C ring was observed (see also **8 xxi** and additional analogues in table S1, Supporting Information).

All the analogues were also tested against *T. brucei* cultured in vitro. These data predominantly followed a trend similar to that of the IC₅₀ data, with decreases in the IC₅₀ values being mirrored by corresponding decreases in the EC₅₀ values. However, there were a few notable exceptions. Compounds **8 iii** (EC₅₀ = 0.48 μM, IC₅₀ = 15.29 μM) and **8 iv** (EC₅₀ = 0.66 μM, IC₅₀ = 7.93 μM) showed much improved EC₅₀ values relative to their corresponding IC₅₀ values. Furthermore, although **8 xiv** (EC₅₀ = 3.64 μM, IC₅₀ > 200 μM) and **8 xv** (EC₅₀ = 4.33 μM, IC₅₀ > 200 μM) were inactive against TryR, they showed reasonable activity against *T. brucei*. These results could suggest that these analogues have additional off-target effects, or are selectively concentrated/metabolically activated, or a combination of these. In addition, all compounds were tested for inhibition of human GR as described in the Experimental Section below. Compounds were tested in duplicate at a single final concentration of 25 μM. All compounds exhibited < 12% inhibition.

Three of the analogues were chosen for assessment of the mode of inhibition with respect to T[S]₂, as described in the Experimental Section. Indatraline (**3**), the original indatraline starting point (Figure 2a), is a linear competitive inhibitor of TryR.

However, compounds **8 ii** (Figure 2b) and **8 iii** were confirmed by an *F*-test to show a mode of mixed inhibition. The mixed inhibition mode indicates that these inhibitors are able to bind to the substrate–enzyme complex as well as to the free enzyme, whilst the competitive mode indicates binding only to the free enzyme. However, even for the mixed mode inhibitors, the *K*' values are approximately fivefold greater than the *K*_i values, suggesting that these compounds bind much more favourably to the free enzyme. This subtle shift in mode of inhibition may represent a useful direction for further development, because mixed inhibitors are less susceptible to the effects of substrate accumulation than competitive inhibitors. For these series, driving down the *K*' value may be more effective than focussing solely on the potency (IC₅₀) of the inhibitors.

Conclusions

We previously reported the screening of the Sigma-LOPAC¹²⁸⁰ collection of bioactive compounds for inhibitors of TryR and have described follow-up studies on one of the hits we obtained from this collection.^[5,7] Herein we report our attempts to progress a second hit obtained in this screen. Indatraline **3**, a CNS-active, nonselective monoamine reuptake inhibitor, provided the drug-like starting point for our studies. Using a range of synthetic routes, novel indatraline analogues were prepared in order to explore structure–activity relationships. Whilst it proved difficult to significantly increase the potency of the original compound as an inhibitor of TryR, some insight into the preferred substituent on the amine and in the two aromatic rings was deduced. In addition, detailed mode of action studies indicated that two of the inhibitors exhibit a mixed mode of inhibition. This interesting observation has led us to further refine the criteria that need to be considered when developing inhibitors of this important enzyme.

Experimental Section

Procedures for the synthesis of analogues of indatraline (**3**)

Unless otherwise stated, starting materials and reagents were obtained from commercial suppliers and were used without further purification. ¹H and ¹³C NMR spectra were measured on a Bruker Avance 300/400 instrument. Chemical shifts are calibrated with reference to the residual proton and carbon resonances of the sol-

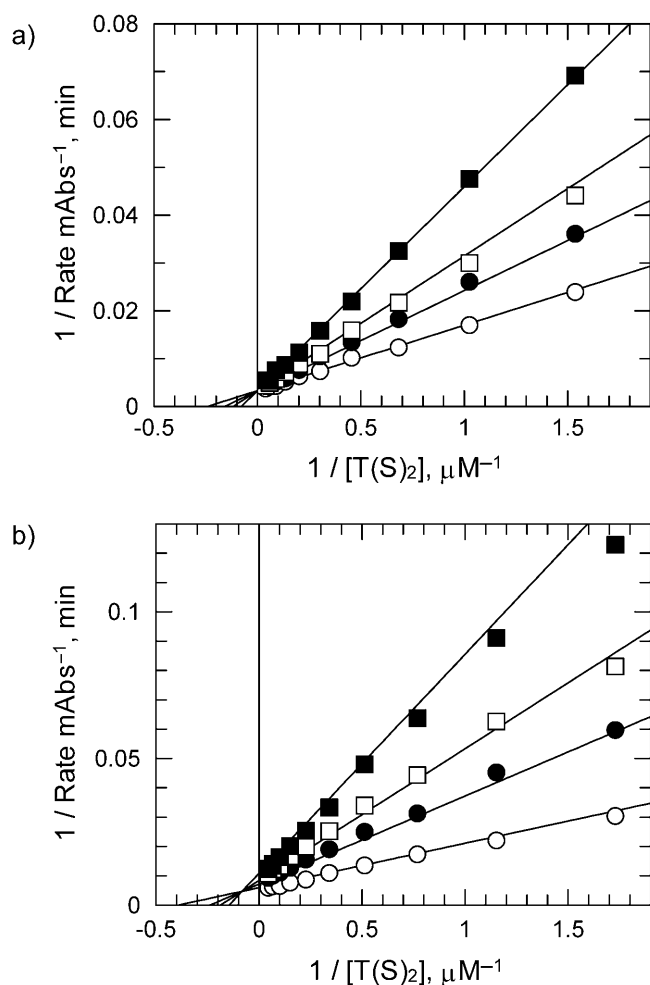


Figure 2. Mode of inhibition by indatraline and compound **8ii**. a) Indatraline: linear competitive inhibition with respect to trypanothione disulfide, $K_i = 5.0 \pm 0.2 \mu\text{M}$. b) **8ii**: linear mixed inhibition, $K_i = 3.5 \pm 0.4 \mu\text{M}$; $K_i' = 16.8 \pm 3.3 \mu\text{M}$.

vent (CDCl_3 : $\delta_{\text{H}} = 7.26$, $\delta_{\text{C}} = 77.0$ ppm). Low- and high-resolution mass spectrometric analyses were recorded using chemical ionisation operating in positive or negative ion mode. For assessment of purity of novel compounds that were tested, see table S2 (Supporting Information).^[11]

General procedure for the formation of amines 8 (Method A): The corresponding amine (0.5 mmol) was added to a solution of **7**^[11] (100 mg, 0.34 mmol) in THF (3 mL), and the solution was stirred at 90 °C in a sealed tube for 4 h. The solvent was removed in vacuo, and the residue partitioned between H_2O (10 mL) and Et_2O (10 mL). The organic layer was extracted with 10% citric acid (3 × 10 mL), and the extract was basified with 10% aqueous NH_4OH solution then extracted with Et_2O (3 × 20 mL). The combined organics were dried (Na_2SO_4) and concentrated in vacuo. Purification by semi-preparative HPLC (Phenomenex Luna silica column, EtOAc/Hex 6:4 (0.1% Et_3NH)) afforded the desired amines.

Trans-[3-(3,4-dichlorophenyl)indan-1-yl]-(3-methylbutyl)amine hydrochloride (8ii**):** Yield 72%, white solid; mp: 190–191 °C; ^1H NMR (400 MHz, MeOD): $\delta = 7.71$ (1H, d, $J = 6.2$ Hz, CH_{Ar}), 7.49 (1H, d, $J = 8.3$ Hz, CH_{Ar}), 7.45–7.43 (2H, m, CH_{Ar}), 7.34 (1H, d, $J = 1.8$ Hz, CH_{Ar}), 7.12–7.06 (2H, m, CH_{Ar}), 4.97–4.95 (1H, m, CH-N), 4.76 (1H, t, $J = 7.6$ Hz, CH-Ar), 3.17–3.13 (2H, m, CH_2N), 2.86–2.81 (1H,

m, CH_{syn}), 2.53–2.48 (1H, m, CH_{anti}), 1.76–1.61 (3H, m, CH and CH_3), 0.99 (3H, d, $J = 6.7$ Hz, CH_3), 0.98 (3H, d, $J = 6.7$ Hz, CH_3); ^{13}C NMR (100 MHz, MeOD): $\delta = 148.8$ (C), 145.9 (C), 138.4 (C), 133.7 (C), 132.0 (CH × 2), 131.9 (C), 131.2 (CH), 129.3 (CH), 129.1 (CH), 127.3 (CH), 127.0 (CH), 63.3 (CH), 49.4 (CH), 45.8 (CH_2), 40.3 (CH_2), 36.1 (CH_2), 27.3 (CH), 22.7 (CH_3), 22.6 (CH_3); LRMS (Cl^+) m/z : 348.1 [$\text{M}+\text{H}$]⁺; HRMS (Cl^+) [$\text{M}+\text{H}$]⁺ m/z expected for $\text{C}_{20}\text{H}_{24}\text{N}^{35}\text{Cl}^{37}\text{Cl}$ 350.1256, obtained 350.1246.

Trans-[3-(3,4-dichlorophenyl)indan-1-yl]octylamine (8iv**):** Yield 60%, colourless oil; ^1H NMR (300 MHz, CDCl_3): $\delta = 7.33$ –7.26 (2H, m, CH_{Ar}), 7.20–7.15 (3H, m, CH_{Ar}), 6.91–6.88 (2H, m, CH_{Ar}), 4.44 (1H, t, $J = 7.6$ Hz, CH-N), 4.28 (1H, dd, $J = 6.8$, 3.4 Hz, CH-Ar), 2.65–2.60 (2H, m, CH_2), 2.41–2.32 (1H, m, CH_{syn}), 2.21–2.12 (1H, m, CH_{anti}), 1.52–1.39 (2H, m, CH_2), 1.28–1.12 (10H, m, $\text{CH}_2 \times 5$), 0.83–0.79 (3H, m, CH_3); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 145.7$ (C), 145.4 (C), 145.4 (C), 132.5 (C), 130.4 (CH), 130.3 (C), 129.9 (CH), 128.5 (CH), 127.4 (CH), 127.3 (CH), 125.3 (CH), 124.8 (CH), 124.8 (CH), 61.9 (CH), 48.5 (CH), 47.5 (CH_2), 43.3 (CH_2), 31.8 (CH_2), 29.8 (CH_2), 29.5 (CH_2), 27.4 (CH_2), 22.7 (CH_2), 14.1 (CH_3); LRMS (Cl^+) m/z : 390.2 [$\text{M}+\text{H}$]⁺; HRMS (Cl^+) [$\text{M}+\text{H}$]⁺ m/z expected for $\text{C}_{23}\text{H}_{30}\text{NCl}_2$ 390.1755, obtained 390.1755.

Trans-4-[3-(3,4-dichlorophenyl)indan-1-yl]morpholine (8vi**):** Yield 61%, colourless oil; ^1H NMR (300 MHz, MeOD): $\delta = 7.80$ –7.77 (1H, m, CH_{Ar}), 7.52–7.43 (3H, m, CH_{Ar}), 7.37 (1H, d, $J = 1.8$ Hz, CH_{Ar}), 7.12 (1H, dd, $J = 8.3$, 1.8 Hz, CH_{Ar}), 7.07–7.04 (1H, m, CH_{Ar}), 5.10 (1H, d, $J = 8.0$ Hz, CH-N), 4.80 (1H, t, $J = 8.0$ Hz, CH-Ar), 4.09–4.04 (2H, m, CH_2), 3.94–3.82 (2H, m, CH_2), 3.50–3.45 (1H, m, CH), 3.37–3.35 (2H, m, CH_2), 3.26–3.06 (2H, m, CH_2 , CH_{syn}), 2.56–2.45 (1H, m, CH_{anti}); ^{13}C NMR (75 MHz, MeOD): $\delta = 150.3$ (C), 145.8 (C), 135.6 (C), 133.7 (C), 132.6 (CH), 132.0 (CH), 131.4 (CH), 129.3 (CH), 129.2 (CH), 128.7 (CH), 127.1 (CH), 72.0 (CH), 65.2 (CH_2), 65.0 (CH_2), 50.8 (CH_2), 50.3 (CH_2), 50.1 (CH), 38.3 (CH_2); LRMS (Cl^+) m/z : 348.1 [$\text{M}+\text{H}$]⁺; HRMS (Cl^+) [$\text{M}+\text{H}$]⁺ m/z expected for $\text{C}_{19}\text{H}_{20}\text{NOCl}_2$ 348.0922, obtained 348.0900.

General procedure for chalcone 13a–i formation: A solution of KOH (6 mmol) in H_2O (3 mL) was slowly added to a solution of ketone **11** (2 mmol) and aldehyde **12** (2 mmol) in EtOH (6 mL) at 0 °C. The reaction mixture was stirred for 2 h, and the resulting precipitate was collected by filtration, washed with EtOH (3 mL), and dried in vacuo. See Supporting Information for analytical data for **13a–i**.

General procedure for the Nazarov reaction (4b,f–i): A solution of **13** (1 mmol) in TFA (4 mL) was heated in a sealed 10 mL tube in a multimode CEM DiscoverTM microwave at 120 °C for 10 min. The powermax mode was enabled, and the microwave was used at its maximum power of 300 W until the set temperature was reached. The solvent was removed in vacuo, and the residue was poured onto ice water and extracted with EtOAc (3 × 10 mL). The combined extracts were washed with saturated NaHCO_3 solution (20 mL) and brine (20 mL), dried (Na_2SO_4) and reduced in vacuo. Purification through a plug of silica (Hex/EtOAc 20:1) afforded the desired indanone. See Supporting Information for analytical data for **4b,f–i**.

General procedure for the formation of alcohols (6b,f–i): NaBH_4 (0.6 mmol) was added to a solution of **4** (0.6 mmol) in MeOH (3 mL), and the reaction was stirred at room temperature for 2 h. A 2 M solution of aqueous KOH (3 mL) was added, and the reaction mixture was extracted with CH_2Cl_2 (3 × 7 mL). The combined organic layers were dried (Na_2SO_4) and reduced in vacuo. Purification of the residue through a plug of silica (Hex/EtOAc 10:1) afforded the

desired alcohol. See Supporting Information for analytical data for **6b,f-i**.

General procedure for the formation of azides **9b,f-i:** Diphenylphosphoryl azide (0.48 mmol) was added to a solution of **6** (0.4 mmol) in anhydrous THF (2 mL) at 0 °C, and the reaction was stirred for 10 min. DBU (0.48 mmol) was slowly added, and the reaction mixture was stirred overnight. H₂O (3 mL) was added, and the reaction mixture was extracted with CH₂Cl₂ (3 × 7 mL). The combined organic layers were dried (Na₂SO₄) and reduced in vacuo. Purification of the residue through a plug of silica (Hex/EtOAc 40:1) afforded the desired azide. See Supporting Information for analytical data for **9b,f-i**.

General procedure for Staudinger reduction to form **10b,f-i:** PS-PPh₃ (0.6 mmol) was added to a solution of **9** (0.3 mmol) in anhydrous THF (5 mL), and the reaction was stirred for 16 h. H₂O (1 mL) was added, and the reaction was stirred for a further 4 h. The reaction mixture was filtered and extracted with CH₂Cl₂ (3 × 5 mL). The combined organic layers were dried (Na₂SO₄) and concentrated in vacuo to give the desired amine **10**. See Supporting Information for analytical data for **10b,f-i**.

General procedure for reductive amination of **10:** The corresponding aldehyde (0.15 mmol) was added to a solution of **10** (0.18 mmol) in anhydrous THF (1 mL), and the solution was stirred for 1 h. Sodium triacetoxyborohydride (0.36 mmol) was added, and the mixture was stirred overnight. A 2 M solution of aqueous KOH (1 mL) was added, and the reaction was extracted with CH₂Cl₂ (3 × 1 mL). The combined organics were dried (Na₂SO₄) and filtered. PS-benzaldehyde resin (0.1 mmol) was added, and the mixture was agitated for 2 h. The resin was removed by filtration, and the solvent was concentrated in vacuo to afford the desired amines.

Trans-[3-(3,4-dichlorophenyl)indan-1-yl]-(4-chlorobenzyl)amine (8viii**):** Yield 54%, colourless oil; ¹H NMR (300 MHz, CDCl₃): δ = 7.35–7.14 (9H, m, CH_{ar}), 6.93–6.86 (2H, m, CH_{ar}), 4.46 (1H, t, *J* = 7.5 Hz, CH-N), 4.32 (1H, dd, *J* = 6.8, 3.5 Hz, CH-Ar), 3.79 (2H, s, CH₂-Ar), 2.44–2.36 (1H, m, CH_{syn}), 2.23–2.14 (1H, m, CH_{anti}); ¹³C NMR (100 MHz, CDCl₃): δ = 145.5 (C), 145.5 (C), 145.1 (C), 138.8 (C), 132.7 (C), 132.5 (C), 130.5 (CH), 130.3 (C), 129.9 (CH), 129.4 (CH × 2), 128.5 (CH × 2), 128.4 (CH), 127.4 (CH), 127.3 (CH), 125.3 (CH), 124.6 (CH), 61.6 (CH), 51.0 (CH₂), 48.5 (CH), 43.8 (CH₂); LRMS (CI⁺) *m/z*: 402.1 [M+H]⁺; HRMS (CI⁺) [M+H]⁺ *m/z* expected for C₂₂H₁₉NCl₃ 402.0583, obtained 402.0573.

Trans-[3-(3,4-dichlorophenyl)indan-1-yl]-(3,4-dichlorobenzyl)amine (8ix**):** Yield 73%, colourless oil; ¹H NMR (300 MHz, CDCl₃): δ = 7.42 (1H, d, *J* = 2.0 Hz, CH_{ar}), 7.34 (1H, dd, *J* = 6.4, 2.6 Hz, CH_{ar}), 7.31 (1H, d, *J* = 8.2 Hz, CH_{ar}), 7.28 (1H, d, *J* = 8.2 Hz, CH_{ar}), 7.22–7.18 (2H, m, CH_{ar}), 7.16–7.12 (2H, m, CH_{ar}), 6.94–6.91 (1H, m, CH_{ar}), 6.88 (1H, dd, *J* = 8.2, 2.0 Hz, CH_{ar}), 4.46 (1H, t, *J* = 7.5 Hz, CH-N), 4.32 (1H, dd, *J* = 6.7, 3.6 Hz, CH-Ar), 3.78 (2H, s, CH₂-Ar), 2.43–2.35 (1H, m, CH_{syn}), 2.24–2.15 (1H, m, CH_{anti}); ¹³C NMR (100 MHz, CDCl₃): δ = 145.4 (C), 145.4 (C), 145.0 (C), 140.8 (C), 132.5 (C), 132.4 (C), 130.8 (C), 130.5 (CH), 130.3 (CH), 129.9 (CH), 129.8 (CH), 129.5 (C), 128.5 (CH), 127.4 (CH), 127.4 (CH), 127.3 (CH), 125.3 (CH), 124.6 (CH), 61.6 (CH), 50.5 (CH₂), 48.5 (CH), 43.8 (CH₂); LRMS (CI⁺) *m/z*: 436.0 [M+H]⁺; HRMS (CI⁺) [M+H]⁺ *m/z* expected for C₂₂H₁₈NCl₄ 436.0193, obtained 436.0194.

Trans-[3-(3,4-dichlorophenyl)indan-1-yl]-(4-methylbenzyl)amine (8x**):** Yield 77%, colourless oil; ¹H NMR (300 MHz, CDCl₃): δ = 7.35–7.34 (1H, m, CH_{ar}), 7.27 (1H, d, *J* = 8.2 Hz, CH_{ar}), 7.19–7.14 (5H, m, CH_{ar}), 7.08–7.06 (2H, m, CH_{ar}), 6.92–6.86 (2H, m, CH_{ar}), 4.46 (1H, t, *J* = 7.5 Hz, CH-N), 4.33 (1H, dd, *J* = 6.8, 3.5 Hz, CH-Ar), 3.78 (2H, s,

CH₂-Ar), 2.45–2.37 (1H, m, CH_{syn}), 2.26 (3H, s, CH₃), 2.22–2.13 (1H, m, CH_{anti}); ¹³C NMR (75 MHz, CDCl₃): δ = 145.6 (C), 145.5 (C), 145.5 (C), 137.2 (C), 136.6 (C), 132.4 (C), 130.4 (CH), 130.2 (C), 129.9 (CH), 129.1 (CH × 2), 128.2 (CH), 128.1 (CH × 2), 127.4 (CH), 127.2 (CH), 125.2 (CH), 124.6 (CH), 61.4 (CH), 51.5 (CH₂), 48.6 (CH), 43.8 (CH₂), 21.1 (CH₃); LRMS (CI⁺) *m/z*: 382.1 [M+H]⁺; HRMS (CI⁺) [M+H]⁺ *m/z* expected for C₂₃H₂₂NCl₂ 382.1129, obtained 382.1134.

Trans-[3-(3,4-dichlorophenyl)indan-1-yl]-(4-methoxybenzyl)amine (8xi**):** Yield 83%, colourless oil; ¹H NMR (300 MHz, CDCl₃): δ = 7.42–7.39 (1H, m, CH_{ar}), 7.35 (1H, d, *J* = 8.3 Hz, CH_{ar}), 7.30–7.22 (5H, m, CH_{ar}), 6.99–6.94 (2H, m, CH_{ar}), 6.91–6.85 (2H, m, CH_{ar}), 4.54 (1H, t, *J* = 7.7 Hz, CH-N), 4.41 (1H, dd, *J* = 6.8, 3.5 Hz, CH-Ar), 3.84 (2H, s, CH₂-Ar), 3.80 (3H, s, CH₃), 2.49 (1H, ddd, *J* = 13.1, 7.7, 3.5 Hz, CH_{syn}), 2.25 (1H, dt, *J* = 13.1, 6.8 Hz, CH_{anti}), 1.69 (1H, brs, NH); ¹³C NMR (75 MHz, CDCl₃): δ = 158.7 (C), 145.6 (C), 145.5 (C), 145.5 (C), 145.3 (C), 132.4 (C), 130.4 (CH), 130.2 (C), 129.9 (CH), 129.3 (CH × 2), 128.2 (CH), 127.4 (CH), 127.2 (CH), 125.2 (CH), 124.6 (CH), 113.8 (CH × 2), 61.5 (CH), 55.3 (CH₃), 51.2 (CH₂), 48.6 (CH), 43.6 (CH₂); LRMS (CI⁺) *m/z*: 398.1 [M+H]⁺; HRMS (CI⁺) [M+H]⁺ *m/z* expected for C₂₃H₂₂NOCl₂ 398.1078, obtained 398.1082.

Trans-[3-(3,4-dichlorophenyl)indan-1-yl]-(4-dimethylaminobenzyl)amine (8xii**):** Yield 68%, colourless oil; ¹H NMR (300 MHz, CDCl₃): δ = 7.46–7.43 (1H, m, CH_{ar}), 7.38 (1H, d, *J* = 8.3 Hz, CH_{ar}), 7.32–7.24 (5H, m, CH_{ar}), 7.02–6.98 (2H, m, CH_{ar}), 6.76 (2H, d, *J* = 8.5 Hz, CH_{ar}), 4.58 (1H, t, *J* = 7.7 Hz, CH-N), 4.46 (1H, dd, *J* = 6.9, 3.3 Hz, CH-Ar), 3.84 (2H, s, CH₂-Ar), 2.97 (6H, s, CH₃ × 2), 2.53 (1H, ddd, *J* = 13.0, 7.7, 3.3 Hz, CH_{syn}), 2.28 (1H, dt, *J* = 13.0, 6.9 Hz, CH_{anti}), 2.15 (1H, brs, NH); ¹³C NMR (75 MHz, CDCl₃): δ = 149.8 (C), 145.6 (C), 145.5 (C), 145.3 (C), 132.4 (C), 130.4 (CH), 130.1 (C), 129.9 (CH), 129.1 (CH × 2), 128.1 (CH), 128.0 (C), 127.4 (CH), 127.2 (CH), 125.1 (CH), 124.6 (CH), 112.7 (CH × 2), 61.3 (CH), 51.3 (CH₂), 48.5 (CH), 43.7 (CH₂), 40.9 (CH₃ × 2); LRMS (CI⁺) *m/z*: 411.1 [M+H]⁺; HRMS (CI⁺) [M+H]⁺ *m/z* expected for C₂₄H₂₅N₂Cl₂ 411.1395, obtained 411.1385.

Trans-[3-(3,4-dichlorophenyl)indan-1-yl]-(4-aminobenzyl)amine (8xiii**):** Yield 76%, colourless oil; ¹H NMR (400 MHz, CDCl₃): δ = 7.41–7.39 (1H, m, CH_{ar}), 7.34 (1H, d, *J* = 8.3 Hz, CH_{ar}), 7.26–7.21 (3H, m, CH_{ar}), 7.15 (2H, d, *J* = 8.4 Hz, CH_{ar}), 6.98–6.95 (2H, m, CH_{ar}), 6.66 (2H, d, *J* = 8.4 Hz, CH_{ar}), 4.54 (1H, t, *J* = 7.8 Hz, CH-N), 4.41 (1H, dd, *J* = 6.9, 3.3 Hz, CH-Ar), 3.78 (2H, d, *J* = 3.0 Hz, CH₂-Ar), 2.49 (1H, ddd, *J* = 13.2, 7.8, 3.3 Hz, CH_{syn}), 2.24 (1H, dt, *J* = 13.2, 6.9 Hz, CH_{anti}), 1.75 (3H, brs, NH × 3); ¹³C NMR (100 MHz, CDCl₃): δ = 149.6 (C), 145.6 (C), 145.5 (C), 145.3 (C), 132.4 (C), 130.4 (CH), 130.1 (C), 129.9 (CH), 129.3 (CH × 2), 128.2 (CH), 128.0 (C), 127.4 (CH), 127.2 (CH), 125.2 (CH), 124.6 (CH), 115.2 (CH × 2), 61.3 (CH), 51.3 (CH₂), 48.6 (CH), 43.7 (CH₂); LRMS (CI⁺) *m/z*: 383.1 [M+H]⁺; HRMS (CI⁺) [M+H]⁺ *m/z* expected for C₂₂H₂₁N₂Cl₂ 383.1082, obtained 383.1084.

Trans-N-[3-(3,4-dichlorophenyl)indan-1-yl]acetamide (8xiv**):** Acetyl chloride (15.6 μL, 0.22 mmol) and DIPEA (115 μL, 0.66 mmol) were added to a solution of **10a** (50 mg, 0.18 mmol) in anhydrous THF (1 mL), and the solution was stirred overnight. A saturated aqueous solution of NaHCO₃ (1 mL) was added, and the reaction was extracted with CH₂Cl₂ (3 × 1 mL). The combined organics were dried (Na₂SO₄) and concentrated in vacuo. Purification through a plug of silica (Hex/EtOAc 5:1) afforded **8xiv** as a white solid (84%); mp: 127–128 °C; ¹H NMR (300 MHz, CDCl₃): δ = 7.42–7.39 (1H, m, CH_{ar}), 7.35 (1H, d, *J* = 8.2 Hz, CH_{ar}), 7.32–7.28 (2H, m, CH_{ar}), 7.19 (1H, d, *J* = 2.1 Hz, CH_{ar}), 7.05–7.02 (1H, m, CH_{ar}), 6.92 (1H, dd, *J* = 8.3, 2.1 Hz, CH_{ar}), 5.67–5.56 (2H, m, NH, CH-N), 4.46 (1H, t, *J* = 7.2 Hz, CH-Ar), 2.52–2.42 (2H, m, CH₂), 2.03 (3H, s, CH₃); LRMS (CI⁺) *m/z*: 320.1 [M+H]⁺; HRMS (CI⁺) [M+H]⁺ *m/z* expected for C₁₇H₁₆Cl₂NO 320.0609, obtained 320.0616.

Trans-N-[3-(3,4-dichlorophenyl)indan-1-yl]-4-methylbenzenesulfonamide (8xv): *p*-Toluenesulfonyl chloride (42 mg, 0.22 mmol) and DIPEA (115 μ L, 0.66 mmol) were added to a solution of **10a** (50 mg, 0.18 mmol) in anhydrous THF (1 mL), and the solution was stirred overnight. A saturated aqueous solution of NaHCO_3 was added, and the reaction was extracted with CH_2Cl_2 (3×1 mL). The combined organics were dried (Na_2SO_4) and concentrated in vacuo. Purification through a plug of silica (Hex/EtOAc 5:1) afforded **8xv** as a white solid (77%); mp: 149–150 $^\circ\text{C}$; ^1H NMR (300 MHz, CDCl_3): δ = 7.82 (2H, d, J = 8.3 Hz, CH_{ar}), 7.36–7.31 (3H, m, CH_{ar}), 7.26–7.23 (2H, m, CH_{ar}), 7.13–7.10 (2H, m, CH_{ar}), 7.00–6.97 (1H, m, CH_{ar}), 6.85 (1H, d, J = 8.3, 2.1 Hz, CH_{ar}), 4.97–4.91 (1H, m, CH-N), 4.65 (1H, d, J = 8.0 Hz, NH), 4.44–4.39 (1H, m, CH-Ar), 2.46 (3H, s, CH_3), 2.44–2.38 (1H, m, CH_{syn}), 2.27–2.18 (1H, m, CH_{anti}); ^{13}C NMR (75 MHz, CDCl_3): δ = 144.9 (C), 144.4 (C), 143.7 (C), 141.9 (C), 137.8 (C), 134.2 (C), 132.6 (C), 130.6 (CH), 129.9 (CH $\times 2$), 129.6 (CH), 129.5 (CH), 129.3 (CH), 128.1 (CH), 127.1 (CH $\times 2$), 125.3 (CH), 124.7 (CH), 57.7 (CH), 47.9 (CH), 44.1 (CH), 21.6 (CH_3); LRMS (Cl^+) m/z expected for $\text{C}_{22}\text{H}_{20}\text{NO}_2\text{Cl}_2\text{S}$ 432.0592, obtained 432.0590.

Trans-[3-(3,4-dichlorophenyl)-6-methoxyindan-1-yl]-(3-methylbutyl)amine (8xvi): Yield (52%), colourless oil; ^1H NMR (300 MHz, CDCl_3): δ = 7.28 (1H, d, J = 8.3 Hz, CH_{ar}), 7.13 (1H, d, J = 2.2 Hz, CH_{ar}), 6.90–6.80 (3H, m, CH_{ar}), 6.71 (1H, dd, J = 8.3, 2.2 Hz, CH_{ar}), 4.36 (1H, t, J = 7.3 Hz, CH-N), 4.25 (1H, dd, J = 6.8, 4.0 Hz, CH-Ar), 3.75 (3H, s, CH_3), 2.67–2.62 (2H, m, CH_2), 2.40–2.32 (1H, m, CH_{syn}), 2.23–2.14 (1H, m, CH_{anti}), 1.58 (1H, sept, J = 6.6 Hz, CH), 1.38–1.31 (2H, m, CH_2), 0.84 (3H, d, J = 6.6 Hz, CH_3), 0.83 (3H, d, J = 6.6 Hz, CH_3); ^{13}C NMR (75 MHz, CDCl_3): δ = 159.5 (C), 144.3 (C), 139.1 (C), 137.4 (C), 132.7 (C), 130.9 (C), 130.6 (CH), 129.9 (CH), 127.3 (CH), 126.3 (CH), 117.9 (CH), 110.9 (CH), 60.9 (CH), 55.7 (CH_2), 48.0 (CH), 42.8 (CH_2), 39.4 (CH_2), 34.4 (CH_2), 26.2 (CH), 22.3 (CH_3), 22.1 (CH_3); LRMS (Cl^+) m/z : 378.1 [$\text{M}+\text{H}$] $^+$; HRMS (Cl^+) [$\text{M}+\text{H}$] $^+$ m/z expected for $\text{C}_{21}\text{H}_{26}\text{Cl}_2\text{NO}$ 378.1391, obtained 378.1399.

Trans-[3-(3,4-dibromophenyl)-6-methoxyindan-1-yl]-(3-methylbutyl)amine (8xvii): Yield (71%), colourless oil; ^1H NMR (300 MHz, CDCl_3): δ = 7.50 (1H, d, J = 8.2 Hz, CH_{ar}), 7.38 (1H, d, J = 2.0 Hz, CH_{ar}), 6.93–6.86 (3H, m, CH_{ar}), 6.79 (1H, dd, J = 8.4, 2.5 Hz, CH_{ar}), 4.42 (1H, t, J = 7.5 Hz, CH-N), 4.31 (1H, dd, J = 6.8, 3.6 Hz, CH-Ar), 3.82 (3H, s, CH_3), 2.73–2.68 (2H, m, CH_2), 2.47–2.39 (1H, m, CH_{syn}), 2.29–2.21 (1H, m, CH_{anti}), 1.64 (1H, sept, J = 6.6 Hz, CH), 1.45–1.37 (2H, m, CH_2), 0.91 (3H, d, J = 6.6 Hz, CH_3), 0.90 (3H, d, J = 6.6 Hz, CH_3); LRMS (Cl^+) m/z : 468.0 [$\text{M}+\text{H}$] $^+$; HRMS (Cl^+) [$\text{M}+\text{H}$] $^+$ m/z expected for $\text{C}_{21}\text{H}_{26}\text{NO}^{79}\text{Br}^{81}\text{Br}$ 468.0361, obtained 468.0356.

Trans-[3-(4-chlorophenyl)-6-methoxyindan-1-yl]-(3-methylbutyl)amine (8xviii): Yield (56%), colourless oil; ^1H NMR (300 MHz, CDCl_3): δ = 7.24–7.22 (2H, m, CH_{ar}), 7.05–6.86 (4H, m, CH_{ar}), 6.77 (1H, dd, J = 8.3, 2.6 Hz, CH_{ar}), 4.45 (1H, t, J = 7.3 Hz, CH-N), 4.31 (1H, dd, J = 7.1, 3.8 Hz, CH-Ar), 3.82 (3H, s, CH_3), 2.72–2.69 (2H, m, CH_2), 2.47–2.38 (1H, m, CH_{syn}), 2.31–2.22 (1H, m, CH_{anti}), 1.62 (1H, sept, J = 6.6 Hz, CH), 1.45–1.38 (2H, m, CH_2), 0.91 (3H, d, J = 6.6 Hz, CH_3), 0.90 (3H, d, J = 6.6 Hz, CH_3); LRMS (Cl^+) m/z : 344.2 [$\text{M}+\text{H}$] $^+$; HRMS (Cl^+) [$\text{M}+\text{H}$] $^+$ m/z expected for $\text{C}_{21}\text{H}_{27}\text{NOCl}$ 344.1781, obtained 344.1784.

Trans-[3-(4-methylphenyl)-6-methoxyindan-1-yl]-(3-methylbutyl)amine (8xix): Yield (73%), colourless oil; ^1H NMR (300 MHz, CDCl_3): δ = 7.05 (2H, d, J = 8.0 Hz, CH_{ar}), 7.01 (2H, d, J = 8.0 Hz, CH_{ar}), 6.97–6.89 (2H, m, CH_{ar}), 6.76 (1H, dd, J = 8.2, 2.4 Hz, CH_{ar}), 4.44 (1H, t, J = 7.4 Hz, CH-N), 4.32 (1H, dd, J = 6.7, 3.7 Hz, CH-Ar), 3.82 (3H, s, CH_3), 2.74–2.70 (2H, m, CH_2), 2.45–2.39 (1H, m, CH_{syn}), 2.31–2.24 (4H, m, CH_3 , CH_{anti}), 1.64 (1H, sept, J = 6.6 Hz, CH), 1.44–

1.39 (2H, m, CH_2), 0.91 (3H, d, J = 6.6 Hz, CH_3), 0.90 (3H, d, J = 6.6 Hz, CH_3); LRMS (Cl^+) m/z : 324.2 [$\text{M}+\text{H}$] $^+$; HRMS (Cl^+) [$\text{M}+\text{H}$] $^+$ m/z expected for $\text{C}_{22}\text{H}_{30}\text{NO}$ 324.2327, obtained 324.2332.

Trans-[3-(4-methoxyphenyl)-6-methoxyindan-1-yl]-(3-methylbutyl)amine (8xx): Yield (76%), colourless oil; ^1H NMR (300 MHz, CDCl_3): δ = 7.22–7.16 (1H, m, CH_{ar}), 6.94–6.91 (2H, m, CH_{ar}), 6.78–6.61 (4H, m, CH_{ar}), 4.45 (1H, t, J = 7.4 Hz, CH-N), 4.33 (1H, dd, J = 6.7, 3.7 Hz, CH-Ar), 3.82 (3H, s, CH_3), 3.75 (3H, s, CH_3), 2.74–2.70 (2H, m, CH_2), 2.46–2.39 (1H, m, CH_{syn}), 2.31–2.26 (1H, m, CH_{anti}), 1.64 (1H, sept, J = 6.6 Hz, CH), 1.44–1.39 (2H, m, CH_2), 0.91 (3H, d, J = 6.6 Hz, CH_3), 0.90 (3H, d, J = 6.6 Hz, CH_3); LRMS (Cl^+) m/z : 340.2 [$\text{M}+\text{H}$] $^+$; HRMS (Cl^+) [$\text{M}+\text{H}$] $^+$ m/z expected for $\text{C}_{22}\text{H}_{30}\text{NO}_2$ 340.2277, obtained 340.2276.

Trans-benzyl-[3-(3,4-dichlorophenyl)-6-methoxyindan-1-yl]amine (8xxi): Yield (59%), colourless oil; ^1H NMR (300 MHz, CDCl_3): δ = 7.32–7.12 (7H, m, CH_{ar}), 6.89–6.81 (3H, m, CH_{ar}), 6.72 (1H, dd, J = 8.3, 2.5 Hz, CH_{ar}), 4.39 (1H, t, J = 7.3 Hz, CH-N), 4.31 (1H, dd, J = 6.8, 4.0 Hz, CH-Ar), 3.82 (2H, s, $\text{CH}_2\text{-Ph}$), 3.75 (3H, s, CH_3), 2.46–2.37 (1H, m, CH_{syn}), 2.24–2.15 (1H, m, CH_{anti}); ^{13}C NMR (75 MHz, CDCl_3): δ = 159.5 (C), 144.4 (C), 144.4 (C), 139.2 (C), 137.5 (C), 132.6 (C), 130.6 (CH), 130.5 (CH $\times 2$), 130.1 (C), 129.9 (CH), 129.1 (CH $\times 2$), 127.4 (CH), 126.3 (CH), 117.9 (CH), 110.8 (CH), 59.7 (CH), 55.7 (CH_3), 47.9 (CH), 47.7 (CH_2), 39.1 (CH_2); LRMS (Cl^+) m/z : 398.1 [$\text{M}+\text{H}$] $^+$; HRMS (Cl^+) [$\text{M}+\text{H}$] $^+$ m/z expected for $\text{C}_{23}\text{H}_{22}\text{NOCl}_2$ 398.1078, obtained 398.1090.

Inhibition of trypanothione reductase: Compounds were tested for inhibition of *T. cruzi* TryR by using a high-throughput microplate assay.^[15] Briefly, assays were set up in 96-well plates using a Biotek Precision 2000 automated liquid handler and initiated with NADPH. The final assay mixtures (0.18 mL) contained TryR (20 mU mL^{-1}), 40 mM HEPES (pH 7.5), 1 mM EDTA, 0.15 mM NADPH, 50 μM DTNB, 6 μM $\text{T}[S]_2$, and inhibitor (100 μM –5 nM in threefold serial dilutions). The rate of TNB $^{\cdot-}$ formation was monitored over 5 min in a Spectramax 340PC plate reader (Molecular Devices) at λ = 412 nm. Raw data were processed with Microsoft Excel. GraFit 5.0 (Erithacus software) was used to fit the data to a three-parameter equation and output the concentration resulting in 50% inhibition (IC_{50} value). Compounds were tested against TryR on three separate occasions, and the IC_{50} values were used to calculate a mean weighted to the standard error. These values are given in Table 1. The mean Z' value throughout the IC_{50} testing was 0.88, indicating suitable assay performance.

Inhibition of glutathione reductase: Compounds were tested for inhibition of human GR by using a modification of the TryR assay method and processed as before. The final conditions in the assay were 7 μM glutathione disulfide, 150 μM NADPH, 50 μM DTNB, and 16 nM human GR.

Assessment of mode of inhibition: Three of the compounds (**3**, **8ii**, and **8iii**) were tested for mode of inhibition with respect to trypanothione. The standard TryR assay was used as before. Aliquots of the assay mixture (180 μL) containing three different concentrations of test compound were added to three rows of a microtitre plate, a fourth row contained only the assay mixture. $\text{T}[S]_2$ was serially diluted across a fifth row of the plate to produce a 12-point range from 500–5.8 μM . The assay was initiated by transferring 20 μL of $\text{T}[S]_2$ row to each of the assay rows. The final 200 μL assay contained 150 μM NADPH, 50 μM DTNB, and 20 mU mL^{-1} TryR and 50–0.58 μM $\text{T}[S]_2$. The final inhibitor concentration in the three rows of the plate ranged from $0.5 \times$ to $3 \times \text{IC}_{50}$. The rate of reaction was measured as before. Each data set was fitted by nonlinear regression to the Michaelis–Menten equation using GraFit 5.0 (Eritha-

cus software). The resulting individual fits were examined as Lineweaver–Burk transformations, and the graphs were inspected for diagnostic inhibition patterns. The entire dataset was then globally fitted to the appropriate equation (competitive, mixed, or uncompetitive inhibition).

Inhibition of *T. brucei* cell proliferation: Compounds were tested for inhibition of proliferation of whole *T. brucei* bloodstream form cells using a microplate assay as previously described.^[16] Cells were cultured in modified HMI-9 media containing 10% FBS and 10% Serum Plus (HMI-9T), in which 0.2 mM 2-mercaptoethanol was replaced with 0.056 mM thioglycerol.^[17] Briefly, test compounds (10 mM in DMSO) were serially diluted in medium in 96-well plates (Greiner). Bloodstream *T. brucei* (S427) cells were added to give a final density of 10^3 cells mL⁻¹ in 0.5% DMSO with inhibitor ranging from 50 to 0.2 μ M in a total volume of 200 μ L. Pentamidine (250–1 nM) was included as a standard drug control on each test plate. Plates were incubated at 37 °C in a 5% CO₂ humidified atmosphere for 72 h, then 45 μ M resazurin (Sigma) was added. After incubation for 4–5 h, fluorescence due to formation of resorufin was measured at λ_{ex} = 528 nm, λ_{em} = 590 nm. Compounds were tested against *T. brucei* cells on three separate occasions, and the EC₅₀ values were used to calculate a mean weighted to the standard error. These values are given in Table 1. The mean Z' value throughout the *T. brucei* testing was 0.67, indicating suitable assay performance.

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- [1] K. Stuart, R. Brun, S. Croft, A. H. Fairlamb, R. E. Gurtler, J. McKerrow, S. Reed, R. Tarleton, *J. Clin. Invest.* **2008**, *118*, 1301–1310.

- [2] a) A. H. Fairlamb, P. Blackburn, P. Ulrich, B. T. Chait, A. Cerami, *Science* **1985**, *227*, 1485–1487; b) A. H. Fairlamb, A. Cerami, *Annu. Rev. Microbiol.* **1992**, *46*, 695–729.
- [3] S. Bailey, K. Smith, A. H. Fairlamb, W. S. Hunter, *Eur. J. Biochem.* **1993**, *213*, 67–75.
- [4] S. Krieger, W. Schwarz, M. R. Ariyanayagam, A. H. Fairlamb, R. L. Krauth-Siegel, C. Clayton, *Mol. Microbiol.* **2002**, *35*, 542–552.
- [5] J. L. Richardson, I. R. E. Nett, D. C. Jones, M. H. Abdille, I. H. Gilbert, A. H. Fairlamb, *ChemMedChem* **2009**, *4*, 1333–1340.
- [6] For a more detailed discussion of this subject, see: C. R. Chong, D. J. Sullivan, Jr., *Nature* **2007**, *448*, 645–646 and references therein.
- [7] S. Patterson, D. C. Jones, E. J. Shanks, J. A. Frearson, I. H. Gilbert, P. G. Wyatt, A. H. Fairlamb, *ChemMedChem* **2009**, *4*, 1341–1353.
- [8] a) K. Bøgesø, A. V. Christensen, J. Hyttel, T. Liljefors, *J. Med. Chem.* **1985**, *28*, 1817–1828; b) J. Hyttel, J. J. Larsen, *J. Neurochem.* **1985**, *44*, 1615–1622; c) S. S. Negus, M. R. Brandt, N. K. Mello, *J. Pharmacol. Exp. Ther.* **1999**, *291*, 60–69.
- [9] C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv. Drug Delivery Rev.* **1997**, *23*, 3–25.
- [10] a) K. P. J. Bøgesø, *J. Med. Chem.* **1983**, *26*, 935–947; b) M. Froimowitz, K.-M. Wu, A. Moussa, R. M. Haidar, J. Jurayj, C. George, E. L. Gardner, *J. Med. Chem.* **2000**, *43*, 4981–4992.
- [11] For a more detailed discussion and information concerning the synthesis of known compounds, see the Supporting Information.
- [12] X.-H. Gu, H. Yu, A. E. Jacobson, R. B. Rothman, C. M. Dersch, C. George, J. L. Flippen-Anderson, K. C. Rice, *J. Med. Chem.* **2000**, *43*, 4868–4876.
- [13] H. Yu, I. J. Kim, J. E. Folk, X. Tian, R. B. Rothman, M. H. Baumann, C. M. Dersch, J. L. Flippen-Anderson, D. Parrish, A. E. Jacobsen, K. C. Rice, *J. Med. Chem.* **2004**, *47*, 2624–2634.
- [14] a) J. G. Topliss, *J. Med. Chem.* **1972**, *15*, 1006–1011; b) J. G. Topliss, *J. Med. Chem.* **1977**, *20*, 463–469.
- [15] C. J. Hamilton, A. Saravanamuthu, I. M. Eggleston, A. H. Fairlamb, *Biochem. J.* **2003**, *369*, 529–537.
- [16] a) D. C. Jones, I. Hallyburton, L. Stojanovski, K. D. Read, J. A. Frearson, A. H. Fairlamb, *Biochem. Pharmacol.* **2010**, *80*, 1478–1486; b) B. Raz, M. Iten, Y. Grether-Bühler, R. Kaminsky, R. Brun, *Acta Trop.* **1997**, *68*, 139–147.
- [17] a) H. Hirumi, K. Hirumi, *J. Parasitol.* **1989**, *75*, 985–989; b) N. Greig, S. Wyllie, S. Patterson, A. H. Fairlamb, *FEBS J.* **2009**, *276*, 376–386.

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